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ATVB in Focus
Platelets Unplugged: Focus on Platelet Biology

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Signaling During Platelet Adhesion and Activation

Zhenyu Li, M. Keegan Delaney, Kelly A. O'Brien, Xiaoping Du

Abstract—Upon vascular injury, platelets are activated by adhesion to adhesive proteins, such as von Willebrand factor and collagen, or by soluble platelet agonists, such as ADP, thrombin, and thromboxane A_2. These adhesive proteins and soluble agonists induce signal transduction via their respective receptors. The various receptor-specific platelet activation signaling pathways converge into common signaling events that stimulate platelet shape change and granule secretion and ultimately induce the "inside-out" signaling process leading to activation of the ligand-binding function of integrin $\alpha_{IIb}\beta_3$. Ligand binding to integrin $\alpha_{IIb}\beta_3$ mediates platelet adhesion and aggregation and triggers "outside-in" signaling, resulting in platelet spreading, additional granule secretion, stabilization of platelet adhesion and aggregation, and clot retraction. It has become increasingly evident that agonist-induced platelet activation signals also cross talk with integrin outside-in signals to regulate platelet responses. Platelet activation involves a series of rapid positive feedback loops that greatly amplify initial activation signals and enable robust platelet recruitment and thrombus stabilization. Recent studies have provided novel insight into the molecular mechanisms of these processes. **(*Arterioscler Thromb Vasc Biol*. 2010;30:2341-2349.)**

Key Words: adhesion molecules ■ G proteins ■ platelets ■ receptors ■ signal transduction

Blood platelets play important roles in hemostasis, thrombosis, wound healing, atherosclerosis, inflammation, immunity, and tumor metastasis.[1–4] Of these functions, the primary physiological function of platelets is to form hemostatic thrombi that prevent blood loss and maintain vascular integrity. This function must be tightly regulated because dysregulated thrombus formation (thrombosis) causes blockage of blood vessels, leading to ischemia. Thrombotic diseases, such as heart attack and ischemic stroke, are a leading cause of mortality in the modern world. Thus, platelets in normal circulation are in a nonadherent "resting" state and become activated at sites of vascular injury after exposure to immobilized adhesive proteins or soluble platelet agonists. The signaling process that occurs during platelet activation can be classified into 3 stages: (1) the interaction of agonists with their respective platelet receptors and receptor-mediated early platelet activation signaling, (2) the intermediate common signaling events, and (3) integrin activation (inside-out signaling) and outside-in signaling. However, platelet activation is a dynamic process involving multiple feedback loops and cross talk between different pathways. In particular, platelets rely on endogenous secondary signal amplification mechanisms and their regulation to achieve a relevant level of response to vascular injury.

In the past 3 decades, remarkable progress has been made in identifying the fundamental mechanisms of platelet function and signaling pathways of platelet activation, which has greatly facilitated the development of antiplatelet therapeutics for preventing and treating thrombotic diseases.[1,2] However, the agents that block fundamental platelet functions, such as integrin blockers, while having potent antithrombotic effects, cause bleeding in approximately 0.5% to 1.5% of patients receiving such compounds. The cyclooxygenase inhibitor (aspirin) and ADP purinergic receptor $P2Y_{12}$ antagonists in use are also associated with problems such as drug resistance and bleeding. A better understanding of intracellular signaling during platelet adhesion and activation will be helpful for the development of new generations of antiplatelet therapies.

Adhesion Receptor–Mediated Platelet Activation and Signaling

Platelet adhesion receptors are the key initiators of platelet activation at sites of vascular injury where platelets become exposed to adhesive proteins in the matrix or on endothelial cells (Figure 1). Interestingly, despite significant differences in their functions and signaling pathways, several major platelet adhesion receptors share many similarities in their signal transduction mechanisms. For example, signal transduction through the glycoprotein Ib–IX-V complex (GPIb-IX), GPVI, and integrins all involve Src family kinases (SFKs), phosphoinositide 3-kinases (PI3Ks), and the immunoreceptor tyrosine-based activation motif (ITAM) signaling pathway.

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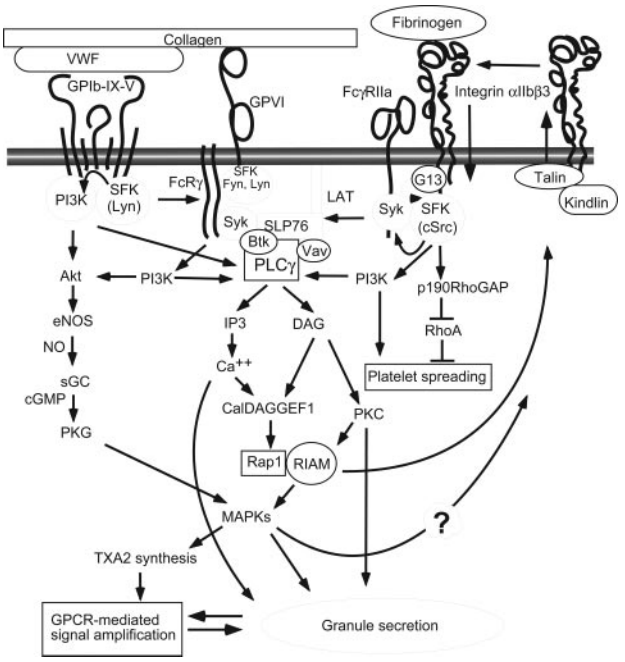

Figure 1. Signaling pathways of 3 major platelet adhesion receptors. sGC, soluble guanylyl cyclase; eNOS, endothelial NO synthase.

Collagen/GPVI-Mediated Platelet Activation Signaling

Platelets express several collagen receptors.[5] Of these receptors, integrin $\alpha_2\beta_1$ is important for platelet adhesion to collagen, whereas GPVI is required for collagen-induced platelet activation. GPVI is a member of the immunoglobulin superfamily and is noncovalently coupled to the Fc receptor γ chain (FcRγ).[6] Upon cross-linking of GPVI by its ligand, the ITAM (a conserved sequence, YxxL/I-$X_{6\ to\ 8}$-XXL/I, originally important in T-cell antigen receptor signaling[7]) within the FcRγ cytoplasmic domain is tyrosine phosphorylated by SFKs (mainly Lyn and Fyn) bound to the cytoplasmic domain of GPVI.[8,9] SFK activation is important for GPVI-mediated platelet activation and involves CD148, a receptorlike protein tyrosine phosphatase that was reported to dephosphorylate the C-terminal inhibitory tyrosines of SFKs.[10]

ITAM phosphorylation leads to binding and activation of the tyrosine kinase Syk, which phosphorylates downstream targets, such as the transmembrane adapter linker for activated T cells (LAT) and the Src homology 2 domain–containing leukocyte phosphoprotein of 76-kDa (SLP-76). This induces the formation of a signaling complex, including LAT, SLP-76, Bruton tyrosine kinase (Btk), Gads, and phospholipase Cγ (PLCγ) 2, which further activates PLCγ2,[11,12] leading to thromboxane A_2 (TXA_2) synthesis, granule secretion, and integrin activation (Figure 1). The pleckstrin homology (PH) domain of PLCγ2 also interacts with the PI3K product phosphatidylinositol 3,4,5-trisphosphate, which facilitates PLCγ2 recruitment to the plasma membrane and activation.[13–15] ITAM signaling is negatively regulated by signals transduced from platelet endothelial cell adhesion molecule-1 (PECAM-1).[16]

von Willebrand Factor/GPIb-IX–Mediated Platelet Activation

Under high shear rate flow conditions present in arteries and arterioles, initial platelet adhesion requires the binding of immobilized von Willebrand factor (VWF) to its platelet receptor, GPIb-IX.[17–19] VWF forms a so-called "catch bond" or "flex bond" with the ligand-binding domain of GPIb-IX,[20,21] allowing transient platelet adhesion under high shear stress. VWF/GPIb-IX interaction also induces platelet activation signaling events, leading to integrin activation and integrin-dependent stable platelet adhesion and aggregation.[19] In addition, GPIb-IX binds thrombin and sensitizes platelets to low-dose thrombin.

There has been evidence that GPIb-IX is associated with the ITAM receptors FcγRIIA[22] or FcRγ.[23] Genetic deletion of ITAM signaling molecules, such as FcRγ, Syk, LAT, SLP-76, and Btk, abolishes the TXA_2 and secretion-dependent second wave of platelet aggregation induced by VWF/botrocetin in washed mouse platelets.[24,25] However, loss of FcRγ and LAT does not appear to affect GPIb-IX–dependent integrin activation and TXA_2 synthesis,[24,26] both of which involve the mitogen-activated protein kinase (MAPK) pathway.[27–29] Similarly, Syk is not required for GPIb-IX– and integrin-dependent stable platelet adhesion to VWF under shear stress.[30] Considering the importance of the ITAM pathway in granule secretion and integrin outside-in signaling, it likely functions as an important signal amplification mechanism in GPIb-IX signaling.

The cytoplasmic domain of the GPIbα chain reportedly interacts with SFKs and PI3Ks,[23,31] which are important for transmitting the "early" activation signals from GPIb-IX,[24,26,31–33] leading to calcium elevation[34] and integrin activation independent of other receptors.[26,30,33] The SFK Lyn is required for activation of PI3K and its downstream effector Akt, leading to integrin activation.[24,30,33] Interestingly, VWF/GPIb-IX interaction induces elevation of intracellular cGMP levels[35,36] and sequential activation of cGMP-dependent protein kinase (PKG) and the MAPKs, p38, and extracellular signal–regulated kinase (ERK).[27,28,35] The cGMP signaling pathway is activated downstream from the Lyn/PI3K/Akt pathway,[30,33] which activates NO synthase. NO may be important for VWF-induced cGMP elevation,[35,36] although SFK-dependent NO-independent soluble guanylyl cyclase activation has been proposed.[37] A role for the PKG/MAPK signaling pathway in GPIb-IX–mediated integrin activation has been shown using inhibitors and knockout mice.[27,28,35] Together, these data reveal that the Lyn/PI3K/Akt/NO/cGMP/PKG/MAPK signaling pathway plays an important role in GPIb-IX–mediated platelet activation. The role of NO and cGMP in platelet activation is biphasic.[35] The low concentrations of NO/cGMP synthesized during platelet activation are stimulatory, whereas high concentrations of NO and cGMP inhibit platelet activation. The biphasic role of the NO/cGMP pathway may serve to stimulate robust hemostatic thrombus formation at sites of vascular injury while preventing overgrowth of the thrombus.

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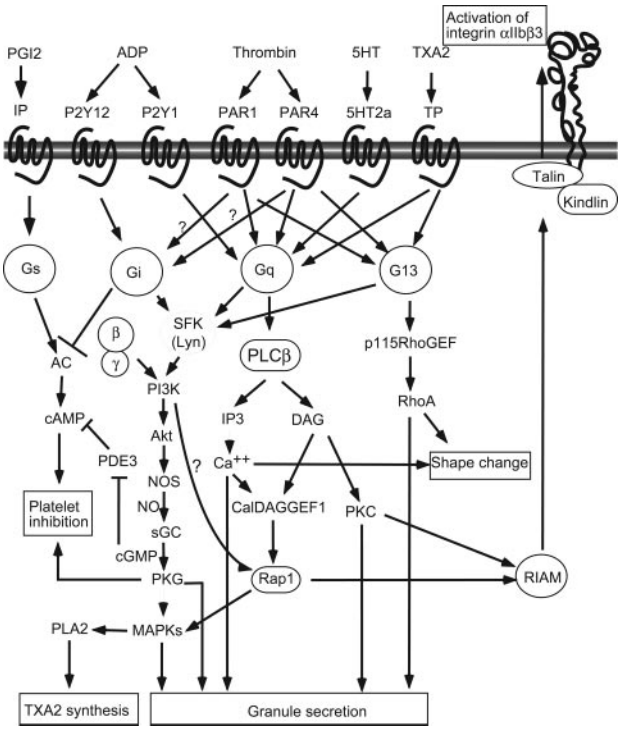

Figure 2. GPCR-coupled platelet activation signaling. PDE3, phosphodiesterase 3; p115RhoGEF, p115 Rho guanine nucleotide exchange factor.

Platelet Activation and Signaling Mediated by G-Protein–Coupled Receptors

A variety of soluble platelet agonists are released from damaged cells (eg, ADP), produced during coagulation (eg, thrombin) and inflammation (eg, platelet-activating factor), and enriched in atherosclerotic plaques (eg, lysophosphatidic acid). They play a critical role in platelet activation and thrombus formation.[38] Equally important, soluble platelet agonists, such as TXA_2, ADP, and serotonin, are released from stimulated platelets that serve to amplify platelet activation and recruit circulating platelets. These agonists activate platelets via G-protein–coupled receptors (GPCRs), a family of 7-transmembrane domain receptors that transmit signals through heterotrimeric G proteins (Figure 2).

The heterotrimeric G proteins consist of 3 subunits (α, β, and γ) that bind to GPCRs in an $\alpha/\beta/\gamma$ complex. On receptor ligation, the α subunit is converted from a GDP-bound form to the active GTP-bound form. Activated Gα subunits dissociate from the receptor, and from the β/γ complex, and interact with specific downstream targets to transmit GPCR signals.[38] The β/γ complex can also interact with and activate downstream effectors, including PI3Kγ.[39] Based on the similarity of α subunits, G proteins can be divided into 4 subfamilies: Gq/G11, G12/G13, Gi/Go/Gz, and Gs, each of which is coupled to selective receptors and downstream effectors (Figure 2).[38] Platelets express Gq, G12/G13, Gi/Gz, and Gs. G proteins in platelets are coupled to agonist receptors that stimulate platelet activation, with the exception of Gs, which is coupled to receptors for physiological platelet inhibitors (prostacyclin and adenosine) that mediate inhibitory signals by stimulating adenylyl cyclase–dependent cAMP synthesis (Figure 2). Thrombin-induced platelet activation is mediated via a dual system of G-protein–coupled protease-activated receptors (PARs): PAR1 and PAR4 in humans[40] and PAR3 and PAR4 in mice.[41] PAR3 appears to sensitize PAR4 to thrombin.[42,43] PAR1 and PAR4 directly couple to Gq and G12/G13[41] and possibly to Gi.[44,45] TXA_2 activates platelets via the TXA_2/prostaglandin H2 receptor (TP), which couples to Gq and G13.[46,47] Serotonin (5-hydroxytryptamine, 5HT) recognizes the Gq-coupled receptor 5HT2A.[38] ADP induces platelet activation via $P2Y_1$ (Gq coupled) and $P2Y_{12}$ (Gi coupled).[38,48] The epinephrine receptor (α2) in platelets is reportedly coupled to Gz, another Gi subtype.[49]

Gq-Mediated Signaling

Gq transmits cellular signals mainly through its interaction and stimulation of PLCβ. Gq signaling is important for GPCR-stimulated platelet granule secretion, integrin activation, and consequent platelet aggregation.[50] Deletion of Gq causes defects in platelet secretion and aggregation in response to a variety of agonists, including thrombin, ADP, TXA_2 analogue U46619, and even collagen (probably because of the dependence of the collagen signaling pathway on TXA_2).[50] In addition, Gq is important in ADP-induced platelet shape change,[50] probably by stimulating calcium/calmodulin- and/or RhoA-dependent contractile signaling.[51]

Gi-Mediated Signaling

Although Gq is required for platelet activation induced by GPCR agonists, it is neither sufficient for platelet aggregation induced by ADP nor for optimal platelet response induced by TXA_2 or low-dose thrombin. The Gi-coupled ADP receptor, $P2Y_{12}$,[52,53] is also required for ADP-induced platelet activation and promotes platelet activation induced by TXA_2 and low-dose thrombin.[45,54] However, it remains controversial whether the thrombin receptors are coupled to Gi directly or indirectly via $P2Y_{12}$.[44,45] The role of Gi-coupled receptors in promoting platelet activation is consistent with the inhibitory effect of Gi on cAMP synthesis, which relieves the inhibitory effect of cAMP-dependent protein kinase on platelet activation. More important, $P2Y_{12}$-coupled Gi is a major mechanism responsible for the activation of PI3K, particularly β/γ subunit–activated PI3Kγ, in platelets[55,56] and subsequent activation of the small GTPase Rap1b,[57,58] a critical mediator of integrin activation.

G13 Signaling

Platelets express both Gα12 and Gα13[59]; however, only Gα13-knockout platelets show reduced and unstable platelet aggregation induced by low-dose thrombin and the TXA_2 analogue U46619. Gα13-knockout platelets have reduced granule secretion that is induced by U46619 but not thrombin.[60] Shape change induced by these agonists is also reduced in Gα13-knockout platelets. GTP-bound Gα13 interacts with and activates guanine nucleotide exchange factors (GEFs) for the small G-protein RhoA, such as p115RhoGEF, which subsequently converts RhoA into the active GTP-bound form.[61] RhoA activates Rho kinase, which phosphorylates and inhibits myosin light chain phosphatase,[62] thus enhancing

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myosin light chain phosphorylation and myosin light chain–dependent contraction. Therefore, G13 stimulates platelet shape change and granule secretion.[62] Interestingly, Gα13 deficiency causes more dramatic defects in platelet adhesion and in hemostasis and thrombosis in vivo, relative to its effects on integrin activation, aggregation, and granule secretion in vitro, suggesting an additional role of Gα13 in platelet function.[60] Indeed, Gα13 binds to the cytoplasmic domain of integrin β_3 and plays a critical role in integrin outside-in signaling.[63]

Common Platelet Activation Signaling and Amplification Pathways

Although the initial signaling mechanisms of various platelet receptors differ, they ultimately converge into common intracellular signaling events. In particular, almost all agonists induce activation of PLC. For example, PLCγ and PLCβ are activated by the ITAM and Gq pathways, respectively.[64] PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to release inositol trisphosphate (IP3) and diacyglycerol (DAG), which activate calcium mobilization and protein kinase C (PKC), respectively. Intracellular calcium and DAG together also activate calcium and DAG-regulated GEF 1 (CalDAG-GEF1), a Rap1 GEF important in integrin signaling.[65]

Calcium Signaling

The critical role of cytosolic calcium in platelet activation and function has been known for many years. Agonist-induced calcium elevation is mainly induced by inositol trisphosphate receptor-mediated release of calcium from intracellular stores and store-operated calcium entry from outside of platelets.[66,67] A role for store-independent calcium entry has also been shown.[66] Canonical transient potential channels and the calcium release–activated channel (Orai1) have been shown to mediate calcium entry.[66,67] Elevation of calcium levels activates multiple signaling events and molecules, including actin-myosin interaction, PKC, calmodulin, NO synthases, and calcium-dependent proteases. Recently, CalDAG-GEFI has mediated several important Ca^{2+} responses, including Rap1 activation, extracellular signal–regulated kinase activation, TXA_2 synthesis, and granule secretion.[67] Calcium elevation also positively regulates SFKs and the PI3K/Akt signaling pathway.[68]

Protein Kinase C

Platelets express several isoforms of the PKC family, including the classical (or conventional) PKC isoforms α and β (calcium and DAG dependent), the novel PKC isoforms δ, θ, and η (DAG dependent and calcium independent), and an atypical PKC isoform ζ (calcium and DAG independent).[69–73] Another novel PKC, PKC ε, is detectable in mouse, but not human platelets.[74] Classical PKCs, particularly PKC α, play a critical and general role in platelet granule secretion and secretion-dependent aggregation. PKC α has also been shown to regulate Rap1 and integrin signaling in a reconstituted Chinese hamster ovary cell model.[75] PKC δ and θ promote dense granule secretion in response to thrombin receptor agonists[69,71,72]; however, their roles in GPVI-mediated secretion and aggregation are controversial. PKC δ has been reported to negatively regulate GPVI-induced dense granule secretion[69,72] or to have no effect.[73] PKC θ has been shown to promote GPVI-dependent platelet granule secretion and aggregation by one group,[71] but to negatively regulate GPVI-mediated granule secretion and platelet activation by other groups.[76,77] Pleckstrin is a major PKC substrate and may possibly be involved in cytoskeleton regulation.[78]

Signals Leading to Granule Secretion and Secretion-Dependent Signal Amplification

A common platelet response to all agonists is the secretion of granule contents. Platelets contain 3 major types of granules: α-granules, containing adhesion proteins (eg, fibrinogen, VWF, coagulation and fibrinolytic factors, cytokines, growth factors, and adhesion receptors); dense granules, containing nucleotides (eg, ADP, ATP, and GTP; serotonin; histamine; pyrophosphates; and divalent cations); and lysozomes, containing a host of proteolytic enzymes.[79] Granule secretion plays critical roles in the amplification of platelet activation, the recruitment of circulating platelets into aggregates, and thrombus stabilization.[79,80] Thus, it can be considered a signaling amplification mechanism. Granule secretion also plays important roles in inflammation, atherosclerosis, host defense, wound healing, angiogenesis, and malignancy.[81] Granule secretion requires fusion between plasma and granule (vesicle) membranes, which is mediated by protein complexes of vesicle-soluble N-ethylmaleimide–sensitive fusion protein attachment receptor (v-SNARE) proteins (mainly vesicle associated membrane protein [VAMP]-8 in platelets) and plasma membrane (target)–SNARE (mainly syntaxin and synaptosome-associated protein-23 in platelets), as reviewed elsewhere.[79] The interaction between SNARE proteins is regulated by their phosphorylation and involves small GTPases, such as Rab27. There are multiple signaling events and pathways that are important in stimulating granule secretion: (1) calcium signaling; (2) PKC-dependent phosphorylation and regulation of SNARE complexes[70]; (3) integrin outside-in signaling; (4) TXA_2 generation, which is important in granule secretion induced by ADP, VWF, and collagen; (5) signaling via the small GTPases Rac-1 and RhoA[82,83]; (6) activation of SFKs, particularly Lyn[84,85]; (7) the PI3K/Akt signaling pathway[56,86–89]; (8) the NO/cGMP/PKG pathway[90,91]; and (9) the signaling pathways of MAPK isoforms p38, ERK, and JNK.[92,93] Recent studies suggest that SFK Lyn activates the PI3K/Akt pathway.[85] PI3K and Akt mediate granule secretion primarily by activating the NO/cGMP/PKG pathway, which stimulates granule secretion through the activation of MAPKs and phosphorylation of SNARE proteins.[90–92]

Integrin Signaling

Inside-Out Signaling

Platelets express integrins $\alpha_{IIb}\beta_3$ (fibrinogen receptor), $\alpha_v\beta_3$ (vitronectin receptor), $\alpha_2\beta_1$ (collagen receptor), $\alpha_5\beta_1$ (fibronectin receptor), and $\alpha_6\beta_1$ (laminin receptor). These integrins share similar signal transduction mechanisms. The most abundant integrin in platelets, $\alpha_{IIb}\beta_3$, is normally kept in a resting or low-affinity state in circulating platelets, but transforms into a high-affinity "activated" state after platelet

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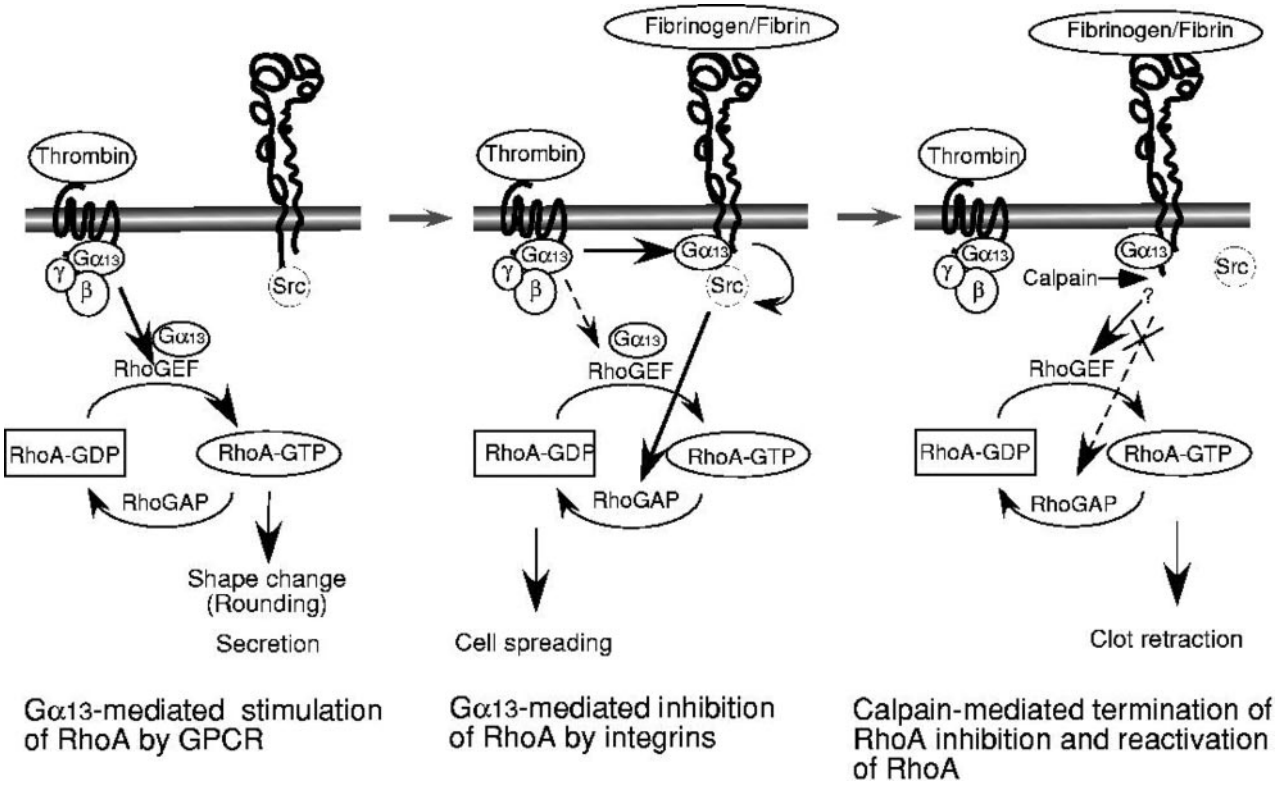

Figure 3. Gα13-dependent cross talk between GPCR signaling and integrin outside-in signaling in regulating RhoA activity in platelets. Data are adapted from Gong et al.[63] RhoGEF, rho guanine nucleotide exchange factor; RhoGAP, rho GTPase-activating protein.

activation. Activated $\alpha_{IIb}\beta_3$, by binding to its ligands (fibrinogen, VWF, and many matrix proteins containing RGD-like sequences), mediates stable platelet adhesion, platelet aggregation, and thrombus formation. The integrin–proximal intracellular signaling mechanism that induces changes in the extracellular ligand binding domain of integrins from a "low-affinity" state to the activated state is referred to as "inside-out" signaling.[1,94] Inside-out signaling requires the binding of talin and kindlins to the cytoplasmic domain of β_3.[95–97] The relationship between talin and kindlins in inside-out signaling is still being clarified. The binding sites in the β_3 cytoplasmic domain for talin and kindlins appear distinct. Talin binds to the membrane proximal region and the NPLY motif of β3,[98–100] whereas kindlins bind to the sequences around the C-terminal NXXY motif.[96,97] Recent studies support the hypothesis that kindlins regulate talin-integrin interaction and cooperate with talin to stimulate inside-out signaling.[97] The binding of talin head domain to β_3 appears to be sufficient to trigger disruption of the interaction between the membrane proximal regions of the cytoplasmic domains of α_{IIb} and β_3 and conformational changes in $\alpha_{IIb}\beta_3$ that propagate to the extracellular ligand-binding domain, transforming integrin $\alpha_{IIb}\beta_3$ into the "active" conformation.[95,101,102] The change of conformation in $\alpha_{IIb}\beta_3$ from a bent to an extended form may result in the activation of the ligand-binding function of the integrin.[1] A possible role of integrin transmembrane domain interactions in this process has also been suggested.[103]

Recent studies suggest that CalDAG-GEF1 and its downstream target, Rap1, play an important role in inside-out signaling,[65,104] providing a possible link between these signaling events and integrin inside-out signaling. CalDAG-GEF1 converts Rap1, a member of the Ras family of small GTPases, from the GDP-bound form to the active GTP-bound form, which interacts with the Rap1-GTP–interacting adaptor molecule (RIAM). The role of CalDAG-GEF1/Rap1 in integrin inside-out signaling is consistent with the data that RIAM promotes $\alpha_{IIb}\beta_3$-talin interaction and integrin activation.[105] The predominant Rap1 isoform expressed in platelets is Rap1b. However, platelets lacking Rap1b[104] or CalDAG-GEF1[65] show only partial defects in $\alpha_{IIb}\beta_3$-dependent platelet aggregation, suggesting that neither Rap1b nor CalDAG-GEFI is fully responsible for inside-out signaling. It remains to be determined whether other isoforms of CalDAG-GEF and Rap1 or alternative pathways are also important in inside-out signaling.

Outside-In Signaling

Ligand binding to integrin $\alpha_{IIb}\beta_3$ mediates platelet adhesion and aggregation and initiates a series of intracellular signaling events ("outside-in" signaling), leading to platelet spreading, granule secretion, stable adhesion, and clot retraction.[106] After ligand binding, integrins undergo "a ligand-induced conformational change" that can be propagated outside-in to the cytoplasmic domain.[107] However, although ligand-induced conformational changes of $\alpha_{IIb}\beta_3$ occur on the binding of multimeric macromolecular ligands, such as fibrinogen, or monomeric peptide ligands, such as RGDS, a significant cellular response only occurs with multimeric macromolecular ligands, suggesting that ligand-induced re-

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ceptor clustering may be important for transmitting outside-in signals. The most proximal signaling event that occurs after integrin ligation is the binding of the G protein subunit Gα13 to the cytoplasmic domain of β_3.[63] The interaction of Gα13 with β_3 stimulates the activation of SFKs,[63] particularly β_3-bound c-Src,[108] thus initiating the SFK-dependent signals required for outside-in signaling. SFKs mediate outside-in signaling through the following mechanisms. (1) SFK-mediated phosphorylation of the 2 NXXY motifs in the cytoplasmic domain of β_3 is critically important for outside-in signaling.[109] Phosphorylation at Y747 negatively regulates talin binding.[110] Phosphorylation at Y759 protects β_3 from calpain cleavage.[111] β_3-Tyrosine phosphorylation may also promote β_3 interaction with intracellular molecules, such as myosin heavy chain and adapter protein SHC.[112] (2) c-Src phosphorylates and activates a major RhoA GTPase-activating protein, p190 Rho GTPase-activating protein, which inactivates RhoA.[113] By this mechanism, the β_3-bound c-Src mediates transient RhoA inhibition during the early phase of platelet spreading on fibrinogen.[63,114] Inhibition of c-Src or deletion of the c-Src binding site in β_3 abolishes integrin-mediated cell spreading in Chinese hamster ovary cells and platelets,[114,115] which can be reversed by RhoA inhibitors, suggesting that c-Src–mediated transient RhoA inhibition is critical for integrin outside-in signaling, leading to platelet spreading. Interestingly, the c-Src–mediated inhibition of RhoA requires the binding of c-Src to a specific site at the C-terminus of β_3 that is sensitive to cleavage by a calcium-dependent protease (calpain).[114] After thrombus formation and coagulation, cleavage of β_3 by calpain at this site abolishes the interaction of c-Src with β_3, which relieves the inhibitory effect of β_3-bound c-Src on RhoA, leading to activation of RhoA and clot retraction.[114] (3) SFKs activate Syk.[108] In human platelets, this can be mediated through phosphorylation of FcγRIIA,[116] which recruits Syk into the integrin signaling complex. Syk activation may also involve its interaction with the β3 cytoplasmic domain.[117] Syk facilitates the assembly of an SLP-76/LAT/Btk/Vav complex that mediates activation of PLCγ2 and subsequent platelet activation events in a manner analogous to the GPVI-mediated ITAM signaling pathway.[116–118]

Cross Talk Between GPCR Signaling and Integrin Outside-In Signaling

Integrin outside-in signaling amplifies platelet responses to GPCR agonists. Conversely, GPCR signaling promotes integrin outside-in signaling. For example, platelet spreading on fibrinogen is greatly enhanced when platelets are treated with GPCR agonists. This is because GPCRs induce integrin activation and directly regulate integrin outside-in signaling. In particular, GPCR-mediated activation of Gα13, although not directly responsible for integrin activation, greatly enhances the interaction of Gα13 with β_3, which is required for outside-in signaling.[63] More important, the GPCR/Gα13 and integrin outside-in signaling pathways coordinate with each other to dynamically regulate RhoA-dependent signaling in platelets. The ability of these 2 signaling pathways to cross talk and dynamically regulate RhoA-dependent signaling is critical for the processes of shape change, granule secretion, spreading, and clot retraction in platelets (Figure 3).

Conclusions

Significant progress has been made in recent years in our understanding of platelet signal transduction during adhesion and activation. Thus, we face an increasingly complex signaling network in platelets and new frontiers to be explored. Many new opportunities for discovery lie in the molecular details of the apparently well-defined signaling pathways. With the goal of fighting thrombotic and hemorrhagic diseases in mind, it is intriguing to know whether further dissection of the molecular mechanisms of integrin signaling may lead to the development of new inhibitors that specifically inhibit outside-in signaling-mediated amplification of platelet activation and platelet recruitment without blocking the ligand-binding function of integrins critically important in hemostasis. Also, the importance of the cross talk between various adhesion receptor signaling pathways and G-protein–coupled signaling pathways is increasingly evident. Understanding the cross talk between these pathways may provide insight into the phenomenon of "resistance" to existing platelet inhibitors and may allow for the development of new therapeutic agents that are more effective in treating thrombosis, with less bleeding side effect. Finally, the elucidation of platelet signaling pathways that contribute to the functions of platelets in events beyond hemostasis and thrombosis, such as those discussed in other articles in this series, may reveal new therapeutic targets for the treatment of disorders such as inflammatory diseases, atherosclerosis, and cancer.

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Disclosures

Dr Du, University of Illinois, Chicago, holds patents relevant to the topic of this review.

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